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SEMI-ANNUAL PROGRESS REPORT

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January 1, 1953  
For period 1, July 1952  
to 1 January 1953.

Contractor: The Johns Hopkins University

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Principal Investigator: Thomas G. Ward, M.D.

Assistants: Frederick A. H. Rice, Ph.D.

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Title of Project: Antigenic Studies on Influenza Virus

Objectives:

To improve immunization against influenza by studying fundamental relationships between cells and virus.

Abstract of Results:

A. Previous to present period:

1. Monovalent influenza virus vaccines were used in a study at Great Lakes where indications were secured that the vaccinated individuals had a slight reduction in the total respiratory disease rate. No influenza occurred during the winter of 1948-1949 and, therefore, the results cannot be evaluated from the point of view of prevention of influenza.
2. Fourteen different strains of influenza virus were checked homologously and heterologously by the following tests:
  - a. Hemagglutination inhibition.
  - b. Mouse neutralization, using live virus as a vaccine.
  - c. Mouse neutralization, using dead virus as a vaccine.
  - d. Antibody producing capacity test (Eddy).
  - e. Egg neutralization test.
  - f. Complement fixation test.

This study indicated that the Thompson strain in influenza virus had the greatest spread of "protectiveness" but that its "invasiveness" was similar to other A strains of virus employed in the protocols.

3. A crystalline receptor substance has been isolated from human lungs, beef lungs and pork lungs. This material contains carbon, hydrogen, oxygen, nitrogen and phosphorus. It is in the crystalline state and the original weight of lung yields about  $10^{-5}$  grams of the final product. It will inhibit a hemagglutination by influenza virus in the order of 0.01 gamma.

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4. Chemical studies, using ionic exchange columns and chromatography, have been utilized in an attempt to understand the chemical nature of the crystalline receptor substance. Antigenic studies carried out during the report period prior to this one have indicated that the material is very likely not antigenic. The concepts of developing a possible inhibitor antiserum, which would combine with the receptor sites of susceptible cells, has had to be reconsidered. The negative character of these findings cannot be considered conclusive, as studies utilizing adjuvants have not been completed.
5. The model system of influenza virus and the chicken red blood cell has been utilized in studying the relation between the virus particle and one type of cell. Radioactive phosphorus has been employed as the indicator system.

The protocol is carried out by adsorption and eluting Lee or PR8 or Thompson influenza virus strains from normal chicken red blood cells and by treating other similar cells with normal allantoic fluid under the same laboratory conditions. If one then adds a constant amount of inorganic radioactive phosphorus to each of the tubes, it is found that the virus treated cells are capable of picking up more of the radioactive phosphorus than the control cells. If one does an experiment and varies the length of time for adsorption and elution he will find that the cells which are treated for approximately 30 minutes are effected more by the PR8 and Thompson viruses than are those cells which are treated for less than 30 minutes. Longer periods of time, that is up to 4 hours, are essentially the same as the cells treated for 30 minutes. On the other hand, the Lee influenza virus requires approximately one hour to reach the limit of the ability of the red blood cells to absorb radioactive phosphorus.

Oddly enough, if one labels the red blood cells in the chick embryo by inoculation of radioactive phosphorus into the yolk of 7 day old embryos, reincubate for 5 days, harvest embryonic red blood cells and then treats these cells with virus and with normal allantoic fluid, he finds that there is no difference between the two with respect to the release of phosphorus from the surface of the cell.

At the moment there is no logical explanation for this phenomenon, because one would suspect that influenza virus acting enzymatically on the surface of a red blood cell should release more inorganic phosphorus than normal allantoic fluid acting on the same receptor sites.

It hardly seems likely that the technic employed is not sensitive enough, but it may be that the employment of a larger number of cells per tube might indicate some difference.

B. Activities during the present period:

1. Further attempts have been made to purify the influenza virus labelled with radioactive phosphorus, but these studies have been hampered by the lack of a high speed preparatory centrifuge. Such a centrifuge is now on order and was promised for delivery last week.
2. During the past six months tissue culture technics have been established and are now being employed as another laboratory tool in the understanding of the relation between the virus and the cell.

The roller tube technic has been established, the types of media have been determined and the laboratory methods have been worked out. Human embryonic, chick embryonic and adult human tissues are being employed.

A technic for staining the tissue growing in the tube and transplanting to coverslips for examination under higher magnification has been completed.

Radioactive phosphorus is being utilized as an indicator system with labelled virus and the unlabelled tissue culture cells on one hand, and on the other, labelled tissue culture cells with unlabelled virus. The two systems are being utilized in an attempt to further understand the relation between the virus and the cell.

Plans for the Future:

**Immediate:** The immediate plans are to continue the tissue culture and virus relationships with radioactive phosphorus as the indicator system, and possibly using other viruses than influenza in an attempt to quantitate these relationships.

**Long Range:** The long range plans are concerned primarily with an attempt to understand the mechanism of the invasion of cells by influenza virus, with a subsequent hope of developing some method for interfering with this phenomenon.

Reports and Publications:

There were no publications during the current report period.